ÖGH Herpetozoa
Austrian Herpetological Society

Herpetozoa 32: 39–44 (2019)
DOI 10.3897/herpetozoa.32.e35576

A skeletochronological estimate of age and growth in a large riparian frog from Madagascar (Anura, Mantellidae, *Mantidactylus*)

Fabio M. Guarino[1], Angelica Crottini[2], Marcello Mezzasalma[3], Jasmin E. Randrianirina[4], Franco Andreone[5]

1 *Dipartimento di Biologia, Università di Napoli Federico II, Via Cinthia 21, I–80126, Naples, Italy.*
2 *CIBIO, Research Centre in Biodiversity and Genetic Resources, InBIO, Universidade do Porto, Campus de Vairão, Rua Padre Armando Quintas, Nº 7, 4485–661 Vairão, Portugal.*
3 *Department of Life Sciences, Natural History Museum, Cromwell Road, London SW5 5BD, UK.*
4 *Parc Botanique et Zoologique de Tsimbazaza, BP 4096, Antananarivo 101, Madagascar.*
5 *Museo Regionale di Scienze Naturali, Via G. Giolitti, 36, I–10123 Torino, Italy.*

http://zoobank.org/2D5D2145-802F-4326-B1FC-1C6022B7B38A

Corresponding author: *Guarino Fabio Maria* (fabio.guarino@unina.it)

Academic editor: *Günter Gollmann* ♦ **Received** 11 November 2018 ♦ **Accepted** 7 January 2019 ♦ **Published** 13 May 2019

Abstract

We characterized the body size (as snout-vent length), age, sexual size dimorphism, and growth rate in a population of one of the larger riparian frog from Madagascar (*Mantidactylus grandidieri*) from a rainforest patch close to Vevembe, SE Madagascar. We identified a significant female-biased sexual size dimorphism. Age was estimated using phalangeal skeletochronology and was significantly higher in females than in males. Modal age class turned out to be 4 years in both sexes but a large percentage of adult females (75%) fell in the 5–6 years-old classes, while no male exceeded 4 years. We here report *M. grandidieri* as a medium-long-lived anuran species. Von Bertalanffy's model showed similar growth trajectories between the sexes although the growth coefficient in females (k = 0.335) was slightly but not significantly higher than in males (k = 0.329).

Key Words

growth curve, longevity, Malagasy herpetofauna, *Mantidactylus* subgenus, phalangeal skeletochronology, sexual size dimorphism, Vevembe

Introduction

Madagascar is renowned for being one of the most important spots in the world for amphibian diversity (Vieites et al. 2009; Perl et al. 2014; Brown et al. 2016) and a model region to study biological evolution and spatial ecology at different taxonomic levels (Mezzasalma et al. 2016; 2017a, b). More than 350 endemic species are known from there (AmphibiaWeb 2018), and another 200 candidate species still need to be thoroughly assessed and possibly described (Glaw and Vences 2007; Vieites et al. 2009; Perl et al. 2014), making this large island one of the priority places for amphibian conservation (Andreone et al. 2005; 2008; Andreone and Randriamahazo 2008a). Unfortunately, a large portion of Madagascan pristine habitats were (and continue to be) subject to increasing environmental alterations which include deforestation, habitat fragmentation, landgrabbing, and human land use intensification (Harper et al. 2007; Allnutt et al. 2008). The majority of Malagasy amphibians live along the east-

Copyright *Fabio M. Guarino et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

ern and northeastern rainforest belt (Brown et al. 2016; Andreone et al. 2018), although comparatively many species occur also in the most arid areas of the west and south of the island (Andreone et al. 2013; Cocca et al. 2018).

Among the endemic frog species of Madagascar only a few are subject to foraging for food and may offer a useful protein income for Malagasy people, especially those living in rural areas. These consist of some large mantellid species of the genus *Mantidactylus* (subgenus *Mantidactylus*), *Boehmantis microtympanum*, and *Boophis goudoti*, and the introduced dicroglossid species *Hoplobatrachus tigerinus*. In particular, some riparian *Mantidactylus* species are regularly hunted by local people for bush-meat purposes (Andreone 2003). Frogs are usually collected overnight when local people also search for other supplementary food, such as crabs, crayfish, eels, tenrecs, frugivore bats, and lemurs. Frogs are generally directly consumed by the collectors and their families, although they are sometimes sold to restaurants where they are cooked and offered under the name of "cuisses de nymphes" (Andreone et al. 2007). As a consequence of this, *Mantidactylus grandidieri*, *M. guttulatus* and a few other closely related lineages of the *Mantidactylus* subgenus which still need to be taxonomically assessed (Glaw and Vences 2007; Vieites et al. 2009; Perl et al. 2014) are among the few Malagasy species bearing a Malagasy common name ("radaka" or "radakabe").

Frogs belonging to the subgenus *Mantidactylus* are crepuscular or nocturnal, riparian and semi-aquatic frogs, with little-known breeding habits. Indeed, the ecology of most amphibian species of Madagascar is still largely under-reported (Andreone and Randriamahazo 2008a, b; Andreone et al. 2012; 2016). So far, skeletochronology has been used to infer some ecological data and formulate hypotheses on age and growth in some Malagasy amphibian species (e.g.: Guarino et al. 1998, 2010; Tessa et al. 2007, 2011, 2017; Andreone et al. 2011). This method represents one of the most reliable tools to estimate demographic life-history traits of amphibians and other vertebrates in the wild (Castanet et al. 1993; Smirina 1994; Sinsch 2015; Sinsch and Dehling 2017), which can provide valuable information for conservation management purposes. The current study aims to use this method to investigate the age structure, body size, sexual size dimorphism and growth rate in a population of *Mantidactylus grandidieri*.

Material and methods

Frogs were searched overnight and collected during a survey carried out in October 2007 in a rainforest patch close to Vevembe (South East of Madagascar, Vondrozo District, Atsimo–Atsinanana Region; coordinates: 22°47'53"S, 47°11'89"E). After capture, each individual was sexed by observation of secondary sexual characters (SSC): females are larger and fatter, while males present much more developed femoral glands. Juveniles were recognised by their smaller size and reduced development of their femoral glands. Our sampling coincided with the beginning of the wet season; this corresponds to the reproductive period when the species is more easily encountered in the field and when SSC are more evident. However, we further confirmed our field sex determination upon the analysis of a few preserved individuals housed in Museo Regionale di Scienze Naturali (MRSN, Torino, Italy). Individuals were measured for their snout-vent length (SVL) using a vernier caliper with an accuracy of 0.1 mm. For this study, we analysed 15 males (SVL range: 63–78 mm), 15 females (SVL range: 74–88 mm) and 4 juveniles (SVL range: 52–58 cm). From each individual the 3rd toe of the left/right foot was clipped at the level of the second phalanx and stored in 75 % ethanol. Standard laboratory procedures of paraffin embedding and sectioning by means of rotary microtome were performed to prepare phalanges for the skeletochronological analysis (Guarino and Erismis 2008; Liu et al. 2012; Sinsch 2015). We removed from each finger the skin and muscle to isolate the second phalanx, which was subsequently decalcified in 5% nitric acid for 1.5–2 h according to the size of the sample. Phalanges were subsequently washed in running tap water for 12 h, dehydrated through a series of graded ethanol baths, cleared with a terpene of natural origin (Bioclear, Bio Optica, Milan, Italy) and embedded with molten paraffin (60 °C). Phalangeal cross-sections (12 µm thick) were obtained using a standard rotative microtome (Reichert–Jung/Leica 2045, Germany) and were stained with Mayer's Hematoxylin for 30 min. Stained sections were examined using a Motic BA340 compound light microscope, equipped with a digital camera.

The count of the lines of arrested growth (LAGs) was performed independently by two of us (FMG and FA) on at least ten sections per individual and without prior knowledge of the SVL and sex of the analysed individual. Based on the microclimatic features of the sampling area, we assumed that one LAG is formed per year during the cooler and dry period (April–October) when temperature, rainfall and food availability decrease. In case of discrepancies between independent LAG counts, the sections were read again until a final consensus was reached. We selected sections with the smallest medullar cavity and widest periosteal bone to reduce the effects of endosteal resorption, which sometimes leads to the total resorption of the innermost LAGs (Castanet et al. 1993). The rate of endosteal resorption was estimated by a back calculation method, comparing the perimeter of the bone outer margin of the smallest (and presumably also youngest) frogs with that of the reversal line (the resorption line between endosteal and periosteal bone) of large-sized frogs (Guarino et al. 2008, 2014). Growth trajectories were estimated using non-linear regression

according to Von Bertalanffy's model, as generally used in demographic studies on amphibians (e.g., Hemelaar 1988; Miaud et al. 1999; Guarino et al. 2011). We used the following general equation: $SVL_t = SVL_\infty (1 - e^{k(t-t0)})$ where SVL_t is the body length at age t; SVL_∞ is the estimated maximum body length; e the base of the natural logarithm; k is the growth coefficient that defines the shape of the curve; t_0 is age at metamorphosis (0.3 years), which in amphibians represents the starting point of the growth interval. We considered 16 mm as the mean size at metamorphosis, which was calculated based on direct observations and by analysing preserved specimens (n = 5) currently stored in the MRSN herpetological collection. SVL_∞, k, and their asymptotic confidence intervals (CI) were calculated using Growth II software (Henderson and Seaby 2006).

Sexual size dimorphism (SSD) was calculated using the size dimorphism index (SDI) (Lovich and Gibbons 1992), as follows: SDI = (mean length of the larger sex / mean length of the smaller sex) ± 1. This index arbitrarily gives +1 if males are larger than females, defining the result as negative, and –1 if females are larger than males, defining the result as positive. Statistical analysis was performed using PAST software version 3.22 (PAST 2013) (Hammer et al. 2001). Significance was set at level of $P < 0.05$.

Results

Well-defined LAGs were observed in all phalangeal cross sections for both adults and juveniles (Fig. 1). Marked remodelling phenomena were found only in the larger-sized, presumably older, individuals. However, the osteometric analysis revealed that the first LAG was completely removed by endosteal resorption in five adults only (all females). As a result, the age estimate in these animals was corrected by adding one LAG to those visible in our sections. False lines, which are incomplete and only faintly hematoxynophilic, were not present in all the examined phalanges and in any case they were easily differentiated from true LAGs. Double lines, which appear as two very closely adjacent twin lines (Castanet et al. 1993), were rarely observed and counted as single LAG according to other studies. Table 1 shows body size values and estimates of individual age, assuming that each LAG corresponds to one year. Estimated age ranged 2–3 years in juveniles, 3–4 years in males and 4–6 years in females. Both SVL and age mean were significantly higher in females than in males (Student's t-test, SVL: $t = -5.027$, $df = 28$, $P < 0.001$; age: $t = -0.459$, $df = 28$, $P < 0.001$). Modal age class was 4 years in both sexes but a large percentage of adult females (75%) fell into the 5 to 6-year-old classes, while no male exceeded 4 years (Table 2). SDI was equal to 0.14 when calculated for 4-year-old class and 0.11 for the whole sampling, respectively. The occurrence in the studied population of 3-year-old males with developed SSC and of 4-year-old females suggests that males reach sexual maturity one year earlier than females. In both sexes the body size was positively and significantly correlated to the age of the individual (Spearman's rank test: males $D = 160$, $r_s = 0.66$, $P = 0.006$; females, $D = 208$, $r_s = 0.60$, $P < 0.01$). The ANCOVAs analysis showed that the difference in body size between sexes was not significant, ($F_{1,28} = 4.02$. $P = 0.052$) using age as a covariate.

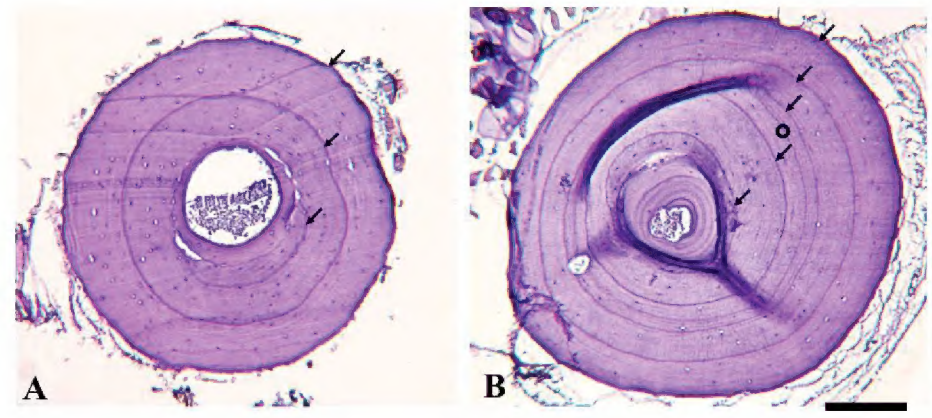

Figure 1. Representative phalangeal cross sections of two individuals of the analysed population of *Mantidactylus grandidieri* from a rainforest close to Vevembe (South East Madagascar). A) Male 63 mm of SVL. B) Female 81 mm of SVL. Arrows indicate lines of arrested growth (LAGs) as identified in this study. In B) the third hematoxynophilic line (empty circle) was interpreted as accessory (intermediate) line caused by a non-annual event. Scale bar: 170 µm.

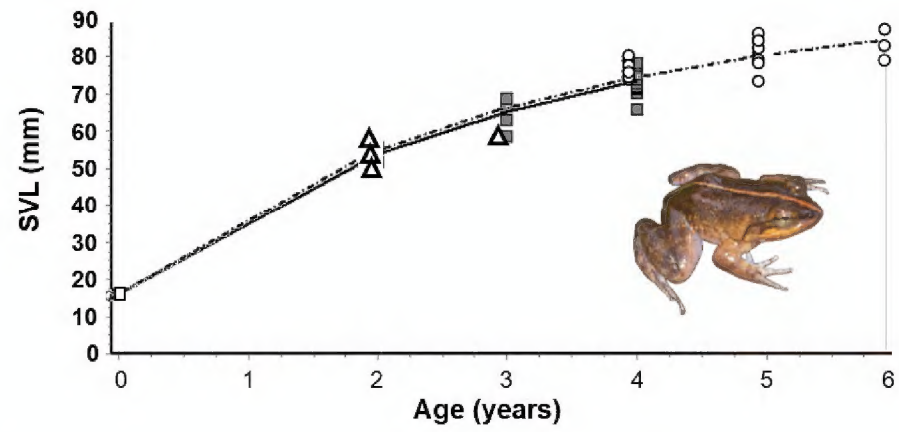

Figure 2. Von Bertalanffy growth curves for males (closed square, black line) and females (empty circle, dotted line) in the analysed population of *Mantidactylus grandidieri* from a rainforest patch close to Vevembe (South East Madagascar). Open triangles were used for juveniles. The empty square represents the frog mean SVL at metamorphosis (here set to 16 mm). On the inset is a picture of an adult *Mantidactylus grandidieri* from the studied population. Growth parameters are reported in the text.

Von Bertalanffy's model showed that the growth trajectories in males and females are identical (Fig. 2) and there is no significant difference between the sexes for the growth coefficient k (males, $k = 0.335 \pm 0{,}024$; females, $k = 0.329 \pm 0.015$). On the other hand, the estimated asymptotic body size of females (SVLasym ± CI: 96.6 ± 1.1 mm) was significantly higher than that of males (SVLasym ± CI: 93.1 ± 2.3 mm).

Table 1. Body size, measured as SVL (in mm) and age (in year, assessed by LAGs counting) of the analysed population of *Mantidactylus grandidieri* from Vevembe. For each parameter, mean ± standard deviation (SD), and min and max value (range) are reported; n, number of studied individuals.

	n		mean± SD	range
Juveniles	4	SVL	55 ± 3	52–58
		Age	2.2 ± 0.1	2–3
Males	15	SVL	72 ± 5	63–78
		Age	3.7 ± 0.4	3–4
Females	15	SVL	79 ± 4	74–88
		Age	4.8 ± 0.7	4–6

Table 2. Number of juveniles, males and females for each age class.

	n	Age class (years)				
		2	3	4	5	6
Juveniles	4	3	1	0	0	0
Males	15	0	4	11	0	0
Females	15	0	0	6	6	3

Discussion

The observed maximal longevity of *M. grandidieri* turned out to be 4 years in males and 6 years in females. This result poses this species in an intermediate position among the Malagasy anurans studied so far (see Guarino et al. 2014; Tessa et al. 2017), being older than the small-size species, such as those belonging to the genus *Mantella* and of *Scaphiophryne gottlebei* (all reaching 1–3 years; Guarino et al. 2010; Andreone et al. 2011; Tessa et al. 2017), but having a shorter observed life span than other species of aproximatively equal body size, such as the tomato frogs (*Dyscophus antongilii* and *D. guineti*) whose males reach 6–7 years and females 11 years (Tessa et al. 2007). In treefrog of *Boophis tsilomaro* males can reach 11 years (females were not studied; Andreone et al. 2002), while in *Boehmantis microtympanum* the maximum observed age was of 7 years (Guarino et al. 1998). Taking into account that *M. grandidieri* probably reaches sexual maturity at 3 years it is surprising that the observed maximum longevity is only of 1–2 years more. Although we do not have empirical data to support this, we cannot exclude that our results might be biased by the intense collecting activity towards larger (and, in general, older) individuals (as observed in Jenkins et al. 2010), thus potentially affecting the age structure of the population that we characterised.

In amphibians, the females mostly exceed the males in body size, with 90% of anurans and 61% of urodeles showing a female-biased dimorphism (Shine 1979; Reinhard et al. 2015). Our study confirmed a sexual dimorphism markedly shifted towards females also in *M. grandidieri*. Large size is likely positively selected in females, being usually correlated with fecundity (larger individuals lay a higher number of eggs), although many amphibians exhibit a female-biased dimorphism in body size regardless of their reproductive mode (Reinhard et al. 2015). Unfortunately, we cannot test such a hypothesis for *M. grandidieri*, since data on its fecundity is currently lacking. The growth patterns exhibited by *M. grandidieri*, with males and females attaining sexual maturity at different ages, suggest a reduced growth after maturity, and indicate that females have a delayed sexual maturity, reaching a larger asymptotic size. This is consistent with the "Model 2" described by Hasumi (2010) for the growth patterns in indeterminate growing vertebrates. In this model, the difference between the sexes on pre-maturational growth can likely result in sexual size dimorphism.The overestimates of the asymptotic SVL may result from the scarcity of small-bodied sexually mature individuals in our data set (Brown et al. 1999).

Further studies are needed to obtain a more exhaustive overview of the age structure trends in Malagasy amphibians. As outlined by Tessa et al. (2017), this could be easily obtained by analysing preserved specimens hosted in museum collections, or even by using clipped toes obtained during other kinds of studies. These data provide valuable information on species life history, which are vital components in order to develop more efficient conservation measures, as stressed in the New Sahonagasy Action Plan for the conservation of the amphibians of Madagascar (Andreone et al. 2016).

Acknowledgements

This work was made possible due to a collaboration between the Museo Regionale di Scienze Naturali (Torino) and the Parc Botanique et Zoologique de Tsimbazaza (Antananarivo). We thank the local administrations of Vevembe Forest for allowing us to perform our survey. A special thank goes to the Malagasy authorities for delivered research and export permits. The work of AC is supported by the Portuguese National Funds through FCT – Foundation for Science and Technology – under the IF/00209/2014/CP1256/CT0011 Exploratory Research Project and the Investigador FCT (IF) grant (IF/00209/2014). JER travel to Vevembe and associated activities were kindly facilitated by M. Vences.

References

Allnutt TF, Ferrier S, Manion G, Powell GVN, Ricketts TH, Fisher BL, Harper GJ, Irwin ME, Kremen C, Labat JN, Lees DC, Pearce TA, Rakotondrainibe F (2008) A method for quantifying biodiversity loss and its application to a 50–year record of deforestation across Madagascar. Conservation Letters 1: 173–181. https://doi.org/10.1111/j.1755–263X.2008.00027.x

AmphibiaWeb (2018) AmphibiaWeb. http://amphibiaweb.org [accessed on 3 November 2018]

Andreone F (2003) Mantellidae: *Mantidactylus*. In: Goodman SM, Benstead JP (Eds) The Natural History of Madagascar. Chicago University Press, Chicago, 910–913.

Andreone F, Bungard M, Freeman K (2007) Threatened amphibians of Madagascar. Museo Regionale Scienze Naturali, Torino, 32 pp.

Andreone F, Carpenter AI, Copsey J, Crottini A, Garcia G, Jenkins RKB, Kohler J, Rabibisoa NHC, Randriamahazo H, Raxworthy CJ (2012) Saving the diverse Malagasy amphibian fauna: where are we four years after implementation of the Sahonagasy Action Plan? Alytes 29: 44–58.

Andreone F, Crottini A, Rosa GM, Rakotoarison A, Scherz MD, Raselimanana AP (2018) Les amphibiens du Nord de Madagascar. Association Vahatra, Antananarivo, 354 pp.

Andreone F, Dawson JS, Rabemananjara FCE, Rabibisoa NHC, Rakotonanahary TS (2016) New Sahonagasy Action Plan 2016–2020. Museo Regionale di Scienze Naturali and Amphibian Survival Alliance, Torino, 46 pp.

Andreone F, Giacoma C, Guarino FM, Mercurio V, Tessa G (2011) Age profile in nine *Mantella* poison frogs from Madagascar, as revealed by skeletochronological analyses. Alytes 27(3): 73–84.

Andreone F, Randriamahazo H (2008a) Essay 5.2. The endemic and threatened amphibians of Madagascar. In: Stuart S, Hoffmann M, Chanson J, Cox N, Berridge R, Ramani P, Young B (Eds) Threatened amphibians of the world. Lynx Editions, Barcelona, 59–60.

Andreone F, Randriamahazo H (2008b) Sahonagasy Action Plan. Conservation Programs for the Amphibians of Madagascar / Programmes de Conservation pour les Amphibiens de Madagascar. Museo Regionale di Scienze Naturali, Conservation International, IUCN / Amphibian Specialist Group, Bogotà, 96 pp.

Andreone F, Rosa GM, Raselimanana AP (2014) Les amphibiens des zones arides de l'Ouest et du Sud de Madagascar. Association Vahatra, Antananarivo, 180 pp.

Andreone F, Vences M, Guarino FM, Glaw F, Randrianirina JE (2002) Natural history and larval morphology of *Boophis occidentalis* (Anura: Mantellidae: Boophinae) provide new insights into the phylogeny and adaptive radiation of endemic Malagasy frogs. Journal of Zoology 257: 425–438. https://doi.org/10.1017/S0952836902001036

Brown J, Sillero N, Glaw F, Bora P, Vieites DR, Vences M (2016) Spatial biodiversity patterns of Madagascar's amphibians and reptiles. PLoS One 2016 11(1): e0144076. https://doi.org/10.1371/journal.pone.0144076.

Brown RP, Znari M, El Mouden EH, Harris P (1999) Estimating asymptotic body size and testing geographic variation in *Agama impalearis*. Ecography 22: 277–283.

Castanet J, Francillon-Vieillot H, Meunier FJ, De Ricqlès A (1993) Bone and individual aging. In: Hall BK (Ed.) Bone (Volume 7). Bone growth. Boca Raton CRC Press, 245–283.

Cocca W, Rosa GM, Andreone F, Aprea G, Eusebio Bergò P, Mattioli F, Mercurio V, Randrianirina, JE, Rosado D, Vences M, Crottini A (2018) The herpetofauna (Amphibia, Crocodylia, Squamata, Testudines) of the Isalo Massif, Southwest Madagascar: combining morphological, molecular and museum data. Salamandra 54(3): 178–200.

Glaw F, Vences M (2007) A Field Guide to the Amphibians and Reptiles of Madagascar. Third edition. Vences and Glaw Verlag, Cologne.

Guarino FM, Andreone F, Angelini F (1998) Growth and longevity by skeletochronological analysis in *Mantidactylus microtympanum*, a rain-forest anuran of southern Madagascar. Copeia 1998(1): 194–198.

Guarino FM, De Pous P, Crottini A, Mezzasalma M, Andreone F (2011) Age structure and growth in a population of *Pelobates varaldii* (Anura, Pelobatidae) from northwestern Morocco. Amphibia-Reptilia 32: 550–556. https://doi.org/10.1163/017353711X605678

Guarino FM, Di Già I, Sindaco R (2008) Age structure by skeletochronology in a declining population of *Rana temporaria* from northern Italy. Acta Zoologica Academiae Scientiarum Hungaricae 54: 99–112.

Guarino FM, Erismis UC (2008) Age determination and growth by skeletochronology of *Rana holtzi*, an endemic frog from Turkey. Italian Journal of Zoology 75: 237–242. https://doi.org/10.1080/11250000701883427

Guarino FM, Garcia G, Andreone F (2014) Huge but moderately long-lived: age structure in *Leptodactylus fallax*, a critically endangered frog from Montserrat, West Indies. Herpetological Journal 24: 167–173.

Guarino FM, Tessa G, Mercurio V, Andreone F (2010) Rapid sexual maturity and short life span in the blue-legged frog and the rainbow frog from the arid Isalo Massif, southern-central Madagascar. Zoology 113: 378–384. https://doi.org/10.1016/j.zool.2010.08.003

Hammer Ø, Harper DAT, Ryan PD (2001) PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica 4(1): 1–9.

Hemelaar A (1988) Age, growth and other population characteristics of *Bufo bufo* from different latitudes and altitudes. Journal of Herpetology 22: 369–388.

Henderson PA, Seaby RM (2001) Growth II. Pisces. Conservation Ltd., Lymington.

Jenkins RKB, Rabearivelo A, Chan CT, Andre WM, Randrianavelona R, Randrianantoandro JC (2009) The harvest of endemic amphibians for food in eastern Madagascar. Tropical Conservation Science 2(1): 25–33. https://doi.org/10.1177/194008290900200105

Lovich JE, Gibbons JW (1992) A review of techniques for quantifying sexual size dimorphism. Growth Development and Aging 56: 269–281.

Liu YH, Zeng Y, Liao WB, Zhou CQ, Mi ZP, Mao M, Chen L (2012) Altitudinal variation in body size in the rice frog (*Rana limnocharis*) in southwestern China. Acta Herpetologica 7: 57–68. https://doi.org/10.13128/Acta_Herpetol–9128

Mezzasalma M, Andreone F, Aprea G, Glaw F, Odierna G, Guarino FM (2017a) Molecular phylogeny, biogeography and chromosome evolution of Malagasy dwarf geckos of the genus *Lygodactylus* (Squamata, Gekkonidae). Zoologica Scripta 46(1): 42–54. https://doi.org/10.1111/zsc.12188

Mezzasalma M, Andreone F, Aprea G, Glaw F, Odierna G, Guarino FM (2017b) When can chromosomes drive speciation? The peculiar case of the Malagasy tomato frogs (genus *Dyscophus*). Zoologischer Anzeiger 268: 41–46. https://doi.org/10.1016/j.jcz.2017.04.006

Mezzasalma M, Andreone F, Glaw F, Petraccioli A, Odierna G, Guarino FM (2016) A karyological study of three typhlopid species with some inferences on chromosome evolution in blindsnakes (Scolecophidia). Zoologischer Anzeiger 264: 34–40. https://doi.org/10.1016/j.jcz.2016.07.001

Miaud C, Guyetant R, Elmberg J (1999) Variations in life-history traits in the common frog *Rana temporaria* (Amphibia: Anura): A literature review and new data from the French Alps. Journal of Zoology London 249: 61–73.

PAST (2013) Data analysis package, version 3.22. Øyvind Hammer, DAT Harper, Oslo.

Perl RGB, Nagy ZT, Sonet G, Glaw F, Wollenberg KC, Vences M (2014) DNA barcoding Madagascar's amphibian fauna. Amphibia-Reptilia 35 197–206. https://doi.org/10.1163/15685381–00002942

Reinhard S, Renner S, Kupfer A (2015) Sexual dimorphism and age of Mediterranean salamanders. Zoology 118: 19–26. https://doi.org/10.1016/j.zool.2014.08.002

Shine R (1979) Sexual selection and sexual dimorphism in the Amphibia. Copeia 1979: 297–306.

Sinsch U (2015) Review: Skeletochronological assessment of demographic life-history traits in amphibians. Herpetological Journal 25: 5–13.

Sinsch U, Dehling JM (2017) Tropical anurans mature early and die young: Evidence from eight Afromontane *Hyperolius* species and a meta-analysis. PLoS ONE 12(2): e0171666. https://doi.org/10.1371/journal.pone.0171666

Smirina EM (1994) Age determination and longevity in amphibians. Gerontology 40: 133–146.

Tessa G, Crottini A, Giacoma C, Guarino FM, Randrianirina JE, Andreone F (2017) Comparative longevity and age at sexual maturity in twelve rainforest frogs of the genera *Boophis, Gephyromantis,* and *Mantidactylus* (Anura: Mantellidae) from Madagascar. Phyllomedusa 16: 13–21. https://doi.org/10.11606/issn.2316–9079.v16i1p13–21

Tessa G, Guarino FM, Giacoma C, Mattioli F, Andreone F (2007) Longevity and body size in three populations of *Dyscophus antongilii* (Microhylidae, Dyscophinae), the tomato frog from north-eastern Madagascar. Acta Herpetologica 2(2): 139–146.

Tessa G, Guarino FM, Randrianirina JE, Andreone F (2011) The age structure in the false tomato frog *Dyscophus guineti* from eastern Madagascar, compared to the closely related *D. antongilii* (Anura, Microhylidae). African Journal of Herpetology 60(1): 84–88. https://doi.org/10.1080/21564574.2011.561881

Vieites DR, Wollenberg KC, Andreone F, Köhler J, Glaw F, Vences M (2009) Vast underestimation of Madagascar's biodiversity evidenced by an integrative amphibian inventory. Proceedings of the National Academy of Sciences of the USA 106: 8267–8272. https://doi.org/10.1073/pnas.0810821106